straight line obtained by joining the two points is produced backwards and cuts the axis at a distance from the origin which represents the amount of piperazine present in the original 1 ml. of the diluted urine (Fig. 1). With the apparatus used in this laboratory all known solutions were found to give results which were 5 μg. too small. A constant correction of 5 μg. was therefore made for each determination.

Dilution of the Urine and Blank Values.—Dilution of the urine 1:25 brings the blank value due to naturally occurring substances within readable limits, and these values are eliminated by the procedure described above. Piperazine standard curves follow Beer's law for samples containing amounts up to 100 μg., and then the curve flattens slightly for amounts up to 200 μg. The amount of piperazine present in the diluted sample must be between 0 and 200 μg. for the actual determination. Higher concentrations of piperazine give rise to rapid precipitation, and, if necessary, the urine must be further diluted.

Colour Reaction.—Upon the addition of the final reagent a reddish-brown colour develops rapidly during the first 20 seconds and reaches its optimum in 10 minutes. After this period the colour increases slowly until precipitation takes place; hence it is essential to take the readings at exactly 10 minutes after the addition of the colour reagent. Reproducible results are given at room temperature (20° C.); higher temperatures increase the colour value in all tubes, and it is therefore necessary to prepare a standard curve appropriate to the temperature of the analytical conditions. The 1:2-naphthoquinone-4-sulphonic acid reagent gives consistent readings with a given quantity of piperazine for a period of two hours after preparation. After this time it slowly darkens and eventually precipitates, giving rise to erroneous results. The recovery of piperazine added to normal urine was found to be satisfactory.

Excretion of Piperazine after Oral Administration in Man

A brief report of the urinary excretion of salts of piperazine by human volunteers was given by Standen *et al.* (1955).

Materials and Methods.—The preparations used were as follows: Piperazine phosphate and piperazine citrate (B.W. & Co.), compressed tablets each containing 500 mg. of piperazine calculated as the hexahydrate. Piperazine adipate ("entacyl"), tablets each containing 251 mg. of piperazine calculated as the hexahydrate. For each excretion study the subject was given tablets equivalent to 3 g. of piperazine hexahydrate together with 300 ml. of water. The dose was given three hours after breakfast. Ten healthy adult volunteers were used for each piperazine salt. In addition excretion rates were determined in four Africans infected with ascarides. Three of these received phosphate and one adipate. Urine was collected at each emptying of the bladder, and the volume and the time were noted. No preservatives were used. Determinations of piperazine were made by the method described, in batches of six samples at a single run. This was found to be the maximum number that could be handled at any one time to keep within the time limit of 10 minutes for development of colour imposed by the method.

Results.—Fig. 2 shows the cumulative excretion of piperazine phosphate, citrate, and adipate. It is clear from these graphs that: (1) there is a wide variation in the rate at which piperazine is excreted by different individuals; (2) the rate of excretion by Africans infected with ascarides was of the same order as the rates in uninfected Europeans; and (3) there was no significant difference between the rates of excretion of piperazine citrate, adipate, and phosphate.

Summary

A method is given for the quantitative analysis of piperazine in urine using 1:2-naphthoquinone-4-sulphonic acid. The method has been used to study the urinary excretion rates of human subjects after oral administration of piperazine phosphate, citrate, and adipate. No significant difference between the rates of excretion of the three salts was observed.

My thanks are due to Dr. L. G. Goodwin and Dr. O. D. Standen for advice and encouragement and also to the volunteers who so willingly participated in the excretion studies.

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ACTION OF NICOTINE ON THE HEART

BY

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The usual view of the action of nicotine is that it is a ganglionic stimulant. Almost all students are taught that Langley painted a solution of nicotine on sympathetic ganglia to discover the anatomical distribution of the cells giving rise to post-ganglionic fibres. When a certain effect, such as pilo-erection, followed the application of nicotine to one ganglion he knew that cells giving rise to fibres which innervated the pilomotor muscles were present in that ganglion.

The increase in heart rate which occurs on smoking a cigarette has usually been explained as a consequence of nicotine stimulating (1) the ganglia of the sympathetic chain from which the nervi accelerantes arise, and (2) perhaps also the cells of the adrenal medulla, liberating adrenaline. If this explanation were correct nicotine would not be expected to have any stimulant action on the isolated heart. The only ganglia believed to be present in the heart itself are the parasympathetic ganglia where the fibres of the vagus make synaptic connexion with the short post-ganglionic fibres. The effect of nicotine on these ganglia would be to slow the heart.

A few years ago, however, our colleague Kottegoda (1953) excised the rabbit heart and separated the atria from the rest of the cardiac tissue, suspending them in a bath so that their contractions could be recorded on a drum. When nicotine was added to the bath he did not observe inhibition, but he observed a mixed effect which was at first inhibitory and then became excitatory. By adding to the bath atropine to exclude the inhibition, he was able to study the quite powerful excitatory action which nicotine then produced. Nicotine caused an increase in rate and amplitude such as is illustrated in Fig. 1 a. The concentrations of nicotine used to produce the effect were, expressed in terms of nicotine base, from 3×10^{-6} g./ml. upwards.

The question then arose to what this stimulant action of nicotine was due. There seemed to be two possi-

bilities. If nicotine was to be regarded as a ganglionic stimulant, then there might be ganglia of the parasympathetic system in the atria which were adrenergic, the post-ganglionic fibres liberating noradrenaline or adrenaline. The second possibility was that nicotine might stimulate chromaffin tissue, which is tissue containing noradrenaline and adrenaline, and might release these amines from it just as it releases them from the adrenal medulla. Several observations have been made that extracts of mammalian hearts contain substances which behave like adrenaline (Shaw, 1938; Cannon and Lissák, 1939; Raab, 1943). von Euler (1946) showed

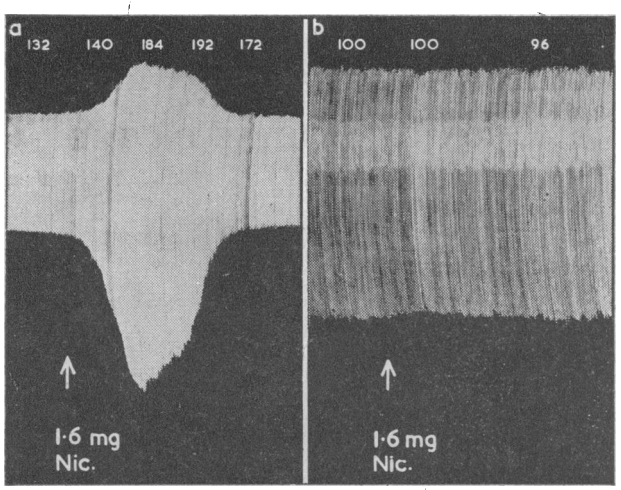

FIG. 1.—Contractions of the isolated atria of the rabbit heart. *a*, Atria from normal rabbit, showing acceleration of rate and amplitude on the addition of 1.6 mg. nicotine acid tartrate to a bath of 40 ml. *b*, Atria from rabbit treated with reserpine, on which the same concentration of nicotine had no action. The figures above the record show the rate per minute.

that the extracts behaved as if they contained noradrenaline, and Goodall (1951) found that in the sheep's heart there was a mean amount of 0.79 μg./g. noradrenaline and 0.15 μg./g. adrenaline. No information was available about the way in which this noradrenaline and adrenaline was held in the heart. It might be intracellular and confer chromaffin properties on the cells.

Action of Reserpine.—Until recently there was no means of shedding further light on the stimulant action of nicotine on the atria. Bertler, Carlsson, and Rosengren (1956) have now shown that when rabbits are injected with reserpine and are left overnight their hearts lose the noradrenaline and adrenaline present within them. Whereas the hearts of normal rabbits contained activity equivalent to a mean figure of 1.57 μg./g. noradrenaline, the rabbits treated with reserpine contained only 0.03 μg./g. We have therefore tested the action of nicotine on the atria of rabbits treated with reserpine.

Method

Bertler, Carlsson, and Rosengren injected rabbits intravenously with the large dose of 5 mg. of reserpine per kg. We found that this injection made the rabbit squeal, and we have therefore given 1.5 mg./kg. by intraperitoneal injection as a preliminary dose on one day, and then followed it 24 hours later with the intravenous injection of 5 mg./kg. Treated in this way the rabbits showed no distress. On the following day the rabbit was killed, the heart was excised, and the atria were suspended in a bath containing McEwen's (1956) solution aerated with O_2+5% CO_2 at 30° C.

Results

The action of nicotine was determined on the atria from five control rabbits and from five rabbits treated with reserpine. The effect of different concentrations of nicotine on one control atrial preparation is shown in Table I.

TABLE I

Concentration of Nicotine (base)	Percentage Increase in Rate	Percentage Increase in Amplitude
$1·5 \times 10^{-6}$ g./ml.	6	16
3·0 ,, ,, ...	12	16
6·0 ,, ,, ...	33	39
1.2×10^{-5} ,, ...	39	162

Since the observations in the other four control preparations were all very similar they are not included. Nicotine did not cause any change in the rate or amplitude of atria from rabbits treated with reserpine, even though it was applied in concentrations up to 5×10^{-5} g./ml. (see Fig. 1 b). These observations showed that the acceleration of the atria caused by nicotine was due to the release of noradrenaline and probably also of small amounts of adrenaline.

Atria from rabbits treated with reserpine not only behaved differently from atria from control rabbits in their response to nicotine but also in the rate and amplitude of their spontaneous beats. A careful comparison was made, using a lever adjusted to magnify contractions to the same extent in different experiments, and, as illustrated in Fig. 1 a and b, the rate of control atria was greater than the rate of atria from reserpine-treated rabbits, while the amplitude of contraction was less. The difference in rate was beyond dispute, the mean spontaneous rates of the two groups being 146 and 112 per minute, this difference being highly significant ($t = 4.55$ and P less than 0.001). We believe also that the difference in amplitude was clear, though admittedly subject to more errors than the difference in rate ($t = 2.98$ and P less than 0.01). The figures for all observations are given in Table II. Finally, it was observed that the increase

TABLE II

Control Atria		Atria from Rabbits treated with Reserpine	
Rate per Minute	Amplitude (mm.)	Rate per Minute	Amplitude (mm.)
132	37	128	60
132	56	136	84
164	77	116	83
168	43	112	73
170	66	100	68
144	10	92	100
136	34	104	64
152	40	108	52
168	43	138	100
		100	70
		104	41
Mean: 146	45	112	72

in rate produced by adding a concentration of adrenaline to the bath was greater in atria from rabbits treated with reserpine than in control atria. During this work we found that the stimulant action of histamine was not diminished in atria from rabbits treated with reserpine (Fig. 2).

Discussion

The evidence obtained by Kottegoda showed that nicotine exerted a stimulating action on the heart itself, and he found that this action was absent in the presence of hexamethonium. This suggested that the stimulant action was exerted on ganglia in the atria, the post-ganglionic fibres of which were adrenergic, and that hexamethonium blocked the effect of nicotine on these ganglia. An alternative explanation was that chromaffin tissue, rather like the tissue of the adrenal medulla, was present in the atria, and that nicotine released noradrenaline and adrenaline from it. The evidence now presented does not allow us to decide between

these alternatives, but it makes clear that in one way or another nicotine stimulates the atria by liberating noradrenaline and adrenaline, since the stimulant action disappears when the rabbit is treated with reserpine and these amines are no longer there.

The presence of noradrenaline and adrenaline in the atria has been known for some time, but, so far as we are aware, there has been no previous evidence suggesting that they play a part in cardiac contraction. Not only is the action of nicotine due to release of these amines, but the spontaneous rate of the isolated atria is modified by them, for in their absence the mean rate was found to fall from 146 to 112.

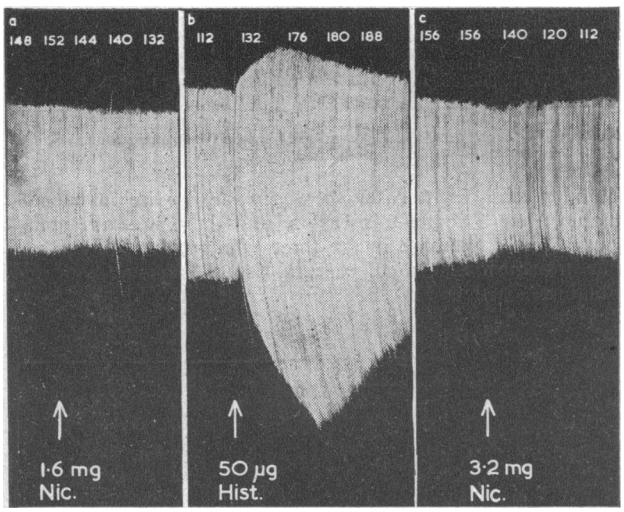

FIG. 2.—Contractions of isolated atria from rabbit treated with reserpine. All observations were made in the presence of atropine. *a* and *c* show that nicotine failed to cause stimulation when 1.6 mg. and 3.2 mg. nicotine acid tartrate were injected. *b* Shows that 50 µg. histamine caused the usual stimulation.

Our evidence has a clear bearing on the question whether a patient who has some cardiac abnormality should smoke. Many doctors, even those who are cardiologists, have been reluctant to tell a patient suffering from cardiac arrhythmias or another disorder that he must not smoke. This reluctance has been due to lack of definite evidence that smoking may do harm. Previous work (Burn, 1951) showed that during smoking the antidiuretic hormone is released from the pituitary posterior lobe, and, since this hormone constricts the coronary vessels, smoking can diminish the coronary blood flow. The present work shows that smoking may liberate noradrenaline and adrenaline from stores within the heart so that they produce acceleration and may cause or exaggerate ventricular arrhythmias.

Summary

Nicotine not only has an inhibitory action on the rate of the isolated heart (as would be expected, since it is a ganglionic stimulant) but it also has a stimulant action which is readily seen when the atria are dissected from other tissue and suspended in a bath which contains atropine to exclude the inhibition.

This stimulant action is shown to be due to nicotine releasing noradrenaline and adrenaline from stores within the heart. These stores can be depleted by giving the rabbit reserpine, and the atria of hearts depleted in this way are not stimulated by nicotine.

The stores of noradrenaline and adrenaline in the heart normally exert some effect and accelerate the spontaneous rate. The mean rate of isolated atria from control animals was 142 per minute, while the mean rate of atria from animals treated with reserpine was 112. The effect of smoking on the heart is discussed.

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INTRAPERITONEAL BLOOD TRANSFUSIONS IN CHILDREN

BY

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The use of the intraperitoneal route for giving blood transfusions is usually mentioned only to be condemned, and most modern textbooks, while describing the technique of intravenous, bone-marrow, and corpus cavernosum transfusions, fail to mention the intraperitoneal route at all. However, the introduction of sterile citrated blood into a healthy peritoneal cavity has been found by previous workers to be of distinct value in raising the haemoglobin and red-cell content of the circulating blood (Siperstein and Sansby, 1923; Cole and Montgomery, 1929; Courtice *et al.*, 1953).

The major advantage of such a transfusion is the ease of administration, a point of considerable importance when dealing with infants and young children. In countries where medical services are as yet incomplete high standards are often impossible, and without skilled medical and nursing supervision intravenous transfusions in infants can be fatal rather than life-saving procedures, overloading of the circulation being the rule rather than the exception.

In an effort to prevent such tragedies with saline infusions, the intraperitoneal route was adopted in the children's wards of King George VI Hospital, Nairobi, three years ago with considerable success (Carter, 1953).

The work of Courtice *et al.* (1953) in investigating the rate of absorption of blood from the peritoneal cavity of cats leaves no doubt whatever that such blood is absorbed intact and constitutes a true transfusion. Earlier workers (Florey and Witts, 1928; Hahn *et al.*, 1944) demonstrated that blood from the peritoneal cavity was absorbed into the thoracic duct, but Courtice *et al.* (1953) have since proved by the simultaneous introduction of cannulae into the thoracic and right lymphatic ducts that there is a greater concentration of absorbed blood in the latter.

A more rapid rate of peritoneal absorption could be obtained by increasing the rate of diaphragmatic movement with the inhalation of 5% carbon dioxide in oxygen (Courtice *et al.*, 1953; Morris, 1953). It was also demonstrated by Courtice and Steinbeck (1951) that the rate of absorption was increased if the pelvis was raised, but that even if the pelvis was in the lowered position injected material was still drawn towards the diaphragm against gravity. These results confirm the earlier investigation of Siperstein and Sansby (1923). It is essential that the same precautions regarding grouping and cross-matching be undertaken as in the case of intravenous transfusions (Wiener, 1943a).